



Comparative Study of Tissue-Specific Esterase Isoenzyme Patterns in Two Freshwater Teleosts: *Labeo rohita* and *Notopterus notopterus* of different orders

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Abstract

The present study investigated the comparative distribution of tissue-specific esterase isoenzyme patterns in two freshwater teleosts, *Labeo rohita* and *Notopterus notopterus*, belonging to different orders. Esterase profiles were examined in the gill, liver, intestine, muscle, brain and eye tissues using electrophoretic separation and inhibitor-sensitivity assays. In *Labeo rohita*, five distinct esterase zones were detected with relative mobility (Rm) values of 0.58, 0.50, 0.41, 0.33 and 0.25. The zones exhibited differential tissue distribution and were classified as carboxylesterase (CE), cholinesterase (ChE), cholinesterase-like enzyme (CHsp), esterases resistant to inhibitors (ER), arylesterase (ArE), and esterase sensitive to organophosphates and pCMB (Esdp). CE and ER esterases showed comparatively broader distribution among the tissues of *L. rohita*. In *Notopterus notopterus*, five esterase zones were observed at Rm values of 0.83, 0.75, 0.66, 0.58 and 0.33. The Rm 0.75 zone showed the widest tissue distribution, occurring in all six tissues, whereas the Rm 0.83 zone was restricted to the liver. The remaining zones displayed distinct tissue-specific patterns and were characterized mainly as CE, ChE and ER esterases, with CHsp also detected in muscle. The inhibitor-sensitivity profiles further demonstrated differences in the biochemical characteristics of individual esterase zones between tissues and species. The findings reveal considerable tissue-specific and species-specific variation in esterase isoenzyme expression in these two freshwater teleosts. Such differential distribution of esterase forms may reflect distinct biochemical functions associated with tissue metabolism and physiological processes. The comparative esterase profiles provide useful biochemical markers for understanding isoenzyme diversity and tissue-specific organization in *L. rohita* and *N. notopterus*.

Keywords: freshwater teleosts, isoenzyme patterns, relative mobility, *L. rohita* and *N. notopterus*.

Introduction

Electrophoretic techniques have become essential for examining protein and enzyme variability among tissues within a species and for comparing corresponding tissues across related organisms (Smith, 1976; Utter, 1985). This method resolves molecules according to their electric charge and size, and polyacrylamide gel electrophoresis is especially useful because its pore size can be adjusted to achieve high-resolution separation of complex protein mixtures (Lakshmipathi, 1994). Esterases (E.C. 3.1.1.x) form a diverse category of hydrolytic enzymes responsible for splitting ester linkages to yield the corresponding acids and alcohols. They are involved in a wide range of physiological roles, such as nerve impulse conduction, reproduction, development, detoxification, and tolerance to xenobiotics (Rao et al., 1998; Hasan et al., 2006). Because they are widely distributed and highly polymorphic, esterases serve as effective biomarkers for monitoring environmental pollution and as useful gene markers in many organisms (Bornscheuer, 2002). The heterogeneity and multiplicity of esterases exceed those of most other enzyme systems, and a single tissue may sometimes contain thirty or more heteromorphic forms (Masters and Holmes, 1975). A striking feature is that homologous tissues from different species, even closely related ones, can differ considerably in both the degree of heterogeneity and the properties of their multiple enzyme forms (Masters and Holmes, 1974). Such variation has proved valuable for taxonomic studies, species discrimination, and understanding evolutionary relationships.

Aldridge (1953) initially separated esterases into 'A' and 'B' types using organophosphate inhibitors, where 'B' esterases are inhibited and 'A' esterases can hydrolyze these compounds. Later, Bergmann et al. (1957) described 'C' esterases that resist organophosphate inhibition. Augustinson (1961) and Holmes and Masters (1967) proposed comprehensive classification schemes based on inhibitor sensitivity, dividing esterases into carboxylesterases (CE, E.C. 3.1.1.1), arylesterases (ArE, E.C. 3.1.1.2), acylesterases (E.C. 3.1.1.6),

and cholinesterases (including acetylcholinesterases E.C. 3.1.1.7 and pseudocholinesterases E.C. 3.1.1.8). Subsequent research, especially on fishes, uncovered esterases with inhibition patterns that did not fit this classification, resulting in the identification of additional categories such as ER esterases (resistant to all inhibitors), Esdp esterases (inhibited by organophosphates and pCMB), Ese esterases (inhibited by eserine alone), and CHsp esterases (inhibited by all three inhibitors) (Hart and Cook, 1976; Haritos and Salamastrakis, 1982; Lakshmipathi and Reddy, 1989).

Initial work on fish esterases concentrated mainly on serum polymorphisms. Nyman (1965) reported inter- and intraspecific differences in serum esterases of the freshwater roach (*Leuciscus rutilus*). Sprague (1967) found polymorphic serum esterases in three Pacific tuna species and linked the variation to multiple alleles within populations. Koehn and Johnson (1967) and Koehn (1969, 1970) showed latitudinal variation in allelic frequencies of serum esterases in catostomid fishes, relating the activity preferences of different alleles to habitat temperature and suggesting that natural selection maintains these polymorphisms. Studies on tissue esterases expanded in the late 1960s. Ecobichon and Israel (1967) characterized esterases in the electric tissue of *Electrophorus*, identifying acetylcholinesterases, pseudocholinesterases, and carboxylesterases. Holmes et al. (1968) carried out comparative investigations across vertebrate tissues, including catfish, laying the groundwork for tissue-specific esterase analysis. Hart and Cook (1976) provided a detailed characterization of tissue esterases in two *Brachydanio* species, described new esterase classes, and demonstrated both tissue and species specificity.

In India, despite its rich ichthyofaunal diversity, electrophoretic studies on fish esterases are still scarce. Rajaiah (1996) conducted a comprehensive study of tissue esterase patterns in thirteen freshwater fishes belonging to the orders Perciformes, Siluriformes, and Atheriniformes. Chatterjee (1989) reported species-specific esterase variation in five *Channa* species.

Nevertheless, comparative studies involving species from different orders remain limited. *Labeo rohita* (Hamilton, 1822), a member of the order Cypriniformes (family Cyprinidae), is one of the most important Indian major carps and a major contributor to freshwater aquaculture. *Notopterus notopterus* (Pallas, 1769), belonging to the order Osteoglossiformes (family Notopteridae), is widely distributed across the Indian subcontinent and possesses distinctive ecological and physiological adaptations. These two species differ markedly in evolutionary history, ecological needs, and physiological traits, making them well suited for comparative biochemical investigations.

The present study was undertaken to: (1) characterize tissue-specific esterase patterns in six tissues (gill, liver, intestine, muscle, brain, and eye) of *L. rohita* and *N. notopterus*; (2) classify individual esterase zones according to their inhibitor sensitivity; (3) compare the distribution of different esterase classes among homologous tissues; and (4) assess the potential of esterase patterns as biochemical markers for species identification and taxonomic studies.

Materials and Methods

Fish Procurement and Laboratory Acclimatization

Fish samples were procured from ponds (tanks) located within a 60 km radius of the Kakatiya University Campus. Capture was performed using nets with the aid of local fishermen. Immediately after collection, the specimens were transferred to the laboratory in plastic buckets containing water from their source ponds. Before the start of experiments, the fish were allowed to adapt to laboratory conditions for about one week in aquaria. Throughout this adaptation phase, they were supplied with natural plankton obtained from their original habitats as feed. This acclimation period was considered essential to minimize stress-related variations in enzyme activity that could arise from capture, handling, and transportation. Only healthy, actively swimming individuals showing no visible signs of

disease or injury were selected for the study. The aquaria were maintained under ambient laboratory conditions of temperature and photoperiod, and water quality was monitored regularly to ensure that the fish remained in a stable physiological state prior to tissue sampling.

Dissection and Tissue Preparation

For tissue extraction, fish were rendered immobile by a blow to the head, and the desired tissues were then removed. Six tissues were selected for the present investigation: gill, liver, intestine, muscle, brain, and eye. These tissues were chosen because they represent diverse physiological functions and metabolic roles, thereby providing a comprehensive picture of esterase distribution across different organ systems. The gill and intestine are directly exposed to the external environment and are involved in respiration and nutrient uptake, respectively, while the liver serves as the principal site of xenobiotic metabolism. Muscle represents a major metabolic tissue, whereas the brain and eye are components of the nervous system in which cholinesterases play critical roles in neurotransmission. Owing to differences in body size, tissues from roughly three large or six small individuals were combined to ensure adequate sample quantity. Pooling of tissues was performed to reduce inter-individual variability and to obtain sufficient protein for reliable electrophoretic analysis. The combined tissues were weighed to the nearest milligram and homogenized in 0.01 M Tris-HCl buffer (pH 7.5) containing 0.9% NaCl. The concentration of tissue homogenates varied according to tissue type as follows: (i) gill – 10%, (ii) liver – 10%, (iii) intestine – 10%, (iv) muscle – 20%, (v) brain – 10%, and (vi) eye – 10%. The buffer pH of 7.5 was selected to approximate physiological conditions and to preserve the catalytic activity and structural integrity of the esterase enzymes during processing. All homogenization steps were carried out in a cold environment to further protect enzyme activity from thermal denaturation.

Centrifugation and Gel Loading

After homogenization, the tissue preparations were placed in ice-jacketed centrifuge tubes to maintain enzyme stability. The homogenates were centrifuged at 2,000 rpm for 10 minutes in a clinical centrifuge at room temperature. This centrifugation step was performed to remove cellular debris, unbroken cells, and other particulate matter, thereby yielding a clear supernatant enriched in soluble proteins. The supernatants obtained were then mixed with an equal volume of 20% sucrose solution containing 0.05% bromophenol blue, which acted as the tracking dye. The sucrose solution served to increase the density of the sample, ensuring that it settled uniformly into the sample wells, while bromophenol blue provided a visible marker to monitor the progress of the electrophoretic run. A 0.1 ml aliquot of this mixture was applied onto the gel for electrophoretic separation of esterase patterns. Care was taken to load equal volumes of each sample so that the resulting banding patterns could be compared reliably across tissues.

Esterase Classification

Esterases were classified according to the procedures of Holmes and Masters (1967), Hart and Cook (1976), Haritos and Salamastrakis (1982), and Lakshmipathi and Reddy (1989), on the basis of their responses to specific inhibitors. Three inhibitors were used in this study: physostigmine (a carbamate), pCMB (a thiol-reactive compound), and paraoxon (an organophosphate). These inhibitors were selected because they act through distinct mechanisms and therefore allow esterases to be differentiated on the basis of their active-site properties and inhibitor sensitivity. Physostigmine specifically targets cholinesterases by binding to the catalytic site, pCMB reacts with sulphydryl groups and thereby inactivates enzymes dependent on cysteine residues for activity, and paraoxon inhibits enzymes possessing a serine hydrolase catalytic mechanism. By comparing the residual enzyme activity after exposure to each inhibitor, individual esterase bands could be assigned to

specific classes. The classification scheme adopted was as follows:

1. **Carboxylesterases (CE):** Esterases inhibited by the organophosphate but not affected by physostigmine or pCMB.
2. **Arylesterases (ArE):** Esterases inhibited by the sulphydryl agent pCMB but not affected by paraoxon or physostigmine.
3. **Cholinesterases (ChE):** Enzymes inhibited by both paraoxon and physostigmine.
4. **ER Esterases:** Enzymes not affected by any of the three inhibitors tested.
5. **Esdp Esterases:** Enzymes inhibited by both pCMB and paraoxon.
6. **Ese Esterases:** Enzymes inhibited by physostigmine alone.
7. **CHsp Esterases:** Enzymes inhibited by paraoxon, physostigmine, and pCMB.

This classification system enabled the identification and characterization of distinct esterase isozymes present in the various tissues examined, and provided a basis for comparing esterase profiles across tissues and species.

Results

Labeo rohita

Esterase Patterns observed in *Labeo rohita*

Gill

The gill tissue displayed three esterase bands on the zymogram with relative mobility (R_m) values of 0.50, 0.41 and 0.25 all exhibiting faint activity. Of these three bands the fractions with R_m 0.50 and 0.25 were identified as ER esterases while the remaining band at R_m 0.41 corresponded to a carboxylesterase (CE) with staining intensity.

Liver

The liver zymogram revealed three esterase zones with R_m values of 0.50, 0.33 and 0.25 all showing enzymatic activity. Among these the bands at R_m 0.50 and 0.33 were characterized as carboxylesterases whereas the band at R_m 0.25 was classified as an ER esterase.

Intestine

Three esterase fractions were detected in the intestine with Rm values of 0.58, 0.50 and 0.33. The bands at Rm 0.50 and 0.33 were identified as ER esterases displaying activity while the fraction at Rm 0.58 was assigned to the Esdp class of esterases.

Muscle

The muscle tissue presented two esterase zones on the zymogram with Rm values of 0.41 and 0.33. The band at Rm 0.41 was identified as a cholinesterase (ChE) and the second band at Rm 0.33 was classified as an esterase.

Brain

Three esterase bands were observed in the brain tissue with Rm values of 0.50, 0.41 and 0.25. The fractions at Rm 0.50 and 0.25 were both characterized as cholinesterases while the band, at Rm 0.41 was identified as an arylesterase (ArE).

Eye

The eye tissue exhibited two esterase zones on the zymogram with Rm values of 0.41 and 0.33. Both fractions were classified as carboxylesterases. Showed moderate enzymatic activity.

Table 1.1:-Inhibitor sensitivity of individual esterase zones in *Labeo rohita*

Name of Tissue	Gill			Liver			Intestine			Muscle		Brain		Eye		
Rm values	.50	.41	.25	.50	.33	.25	.58	.50	.33	.41	.33	.50	.41	.25	.41	.33
Activity	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++	++
pCMB	+	+	+	+	+	+	-	+	+	+	-	+	-	+	+	+
Eserine	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+
Paraaxon	+	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-
Classification	ER	CE	ER	CE	CE	ER	Esdp	ER	ER	ChE	CHsp	ChE	ArE	ChE	CE	CE

Rm = Relative mobility is calculated as a fraction of the distance migrated by the zone from the origin of a tracking dye.

CE = Carboxylesterase; ChE= Cholinesterase; CHsp = Cholinesterase like enzymes; ER= Esterases resistant to inhibitors; ArE = Arylesterases;

Esdp = Esterase sensitive to organophosphates and pCMB

Ese = Esterases sensitive to eserine alone;

+++ = High activity; ++ = Moderate activity; += Low activity; + = Very low activity;

Table 1.2:- Tissue specific distribution of esterases in *Labeo rohita*

Rm values Tissues	1 .58	2 .50	3 .41	4 .33	5 .25
1) Gill		+ ER	+ CE		+ ER
2) Liver		++ CE		++ CE	++ ER
3) Intestine	++ Esdp	++ ER		++ ER	
4) Muscle			++ ChE	++ CHsp	
5) Brain		++ ChE	++ ArE		++ ChE
6) Eye			++ CE	++ CE	

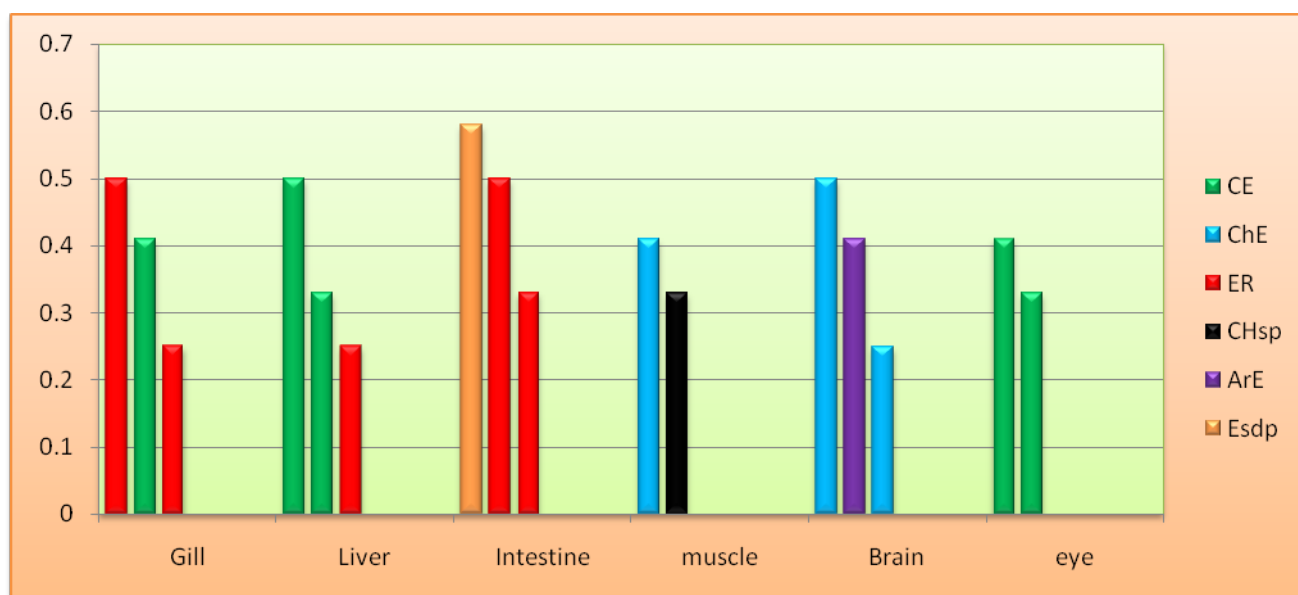
Rm = Relative mobility is calculated as a fraction of the distance migrated by the zone from the origin of a tracking dye.

CE = Carboxylesterase; ChE= Cholinesterase; CHsp = Cholinesterase like enzymes; ER= Esterases resistant to inhibitors; ArE = Arylesterases;

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Ese = Esterases sensitive to eserine alone;

+++ = High activity; ++ = Moderate activity; += Low activity; + = Very low

Figure 1. Tissue specific distribution of esterases in *Labeo rohita*

Notopterus notopterus

Esterase Patterns observed in *Notopterus notopterus*

Gill

Notopterus notopterus gill tissue zymogram was found to have three active esterase fractions with

Rm values of 0.75, 0.58 and 0.33. The fractions with Rm values of 0.58 and 0.33 were inhibited by eserine and paraoxon and hence were termed as cholinesterases (ChE). The other fraction with an Rm value of 0.75 was not affected by the inhibitors and thus classified as ER esterase with moderate activity level.

Liver

The liver tissue had four active esterase fractions with Rm values of 0.83, 0.75, 0.66 and 0.33. The two fractions with Rm values of 0.75 and 0.66 were found to be carboxylesterase (CE) and ER esterase with moderate activity level respectively. The two other fractions with Rm values of 0.83 and 0.33 were found to be ER and ChE esterases with moderate activity levels respectively.

Intestine

There were three active esterase fractions found from the intestine zymogram with Rm values of 0.75, 0.66 and 0.33 representing CE, ChE and ER esterases respectively. Out of these three fractions, the two fractions with Rm values of 0.66 and 0.33 were found to have high activity levels while the other fraction with Rm value of 0.75 had moderate activity level.

Muscle

There were two active esterase fractions in the muscle tissue with Rm values of 0.75 and 0.33

which were found to be CE and CHsp esterases respectively with moderate activity levels.

Brain

The brain tissue had two active esterase fractions with Rm values of 0.75 and 0.58. Out of these two fractions, the one with an Rm value of 0.75 remained unaffected by all the three inhibitors and thus was classified as ER esterase. The other fraction with an Rm value of 0.58 was found to be cholinesterase (ChE).

Eye

The eye tissue had two active esterase fractions with Rm values of 0.75 and 0.58. The fraction with an Rm value of 0.75 was found to be inhibited by paraoxon only and thus was classified as a carboxylesterase (CE). The other fraction with an Rm value of 0.58 was inhibited by paraoxon and eserine and thus was a cholinesterase (ChE) fraction with moderate activity level.

Table 2.1:- Inhibitor sensitivity of individual esterase zones in *Notopterus notopterus*

Name of Tissue	Gill			Liver				Intestine			Muscle		Brain		Eye	
Rm values	.75	.58	.33	.83	.75	.66	.33	.75	.66	.33	.75	.33	.75	.58	.75	.58
Activity	++ +	++	++	++	++	++	++	+++	+++	+++	+++	++	++ +	++	++ +	++
pCMB	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Eserine	+	-	-	+	+	+	-	+	-	+	+	-	+	-	+	-
Paraoxon	+	-	-	+	-	+	-	-	-	+	-	-	+	-	-	-
Classification	ER	ChE	ChE	ER	CE	ER	ChE	CE	ChE	ER	CE	CHsp	ER	ChE	CE	ChE

Rm = Relative mobility is calculated as a fraction of the distance migrated by the zone from the origin of a tracking dye.

CE = Carboxylesterase; ChE= Cholinesterase; CHsp = Cholinesterase like enzymes; ER= Esterases resistant to inhibitors; ArE = Arylesterases;

Esdp = Esterase sensitive to organophosphates and pCMB

Ese = Esterases sensitive to eserine alone;

+++ = High activity; ++ = Moderate activity; += Low activity; + = Very low activity;

Table 2.2:- Tissue specific distribution of esterases in *Notopterus notopterus*

Rm values	1	2	3	4	5
Tissues	.83	.75	.66	.58	.33
1) Gill		+++ ER		++ ChE	++ ChE
2) Liver	++ ER	++ CE	++ ER		++ ChE
3) Intestine		+++ CE	+++ ChE		+++ ER
4) Muscle		+++ CE			++ CHsp
5) Brain		+++ ER		++ ChE	
6) Eye		+++ CE		++ ChE	

Rm = Relative mobility is calculated as a fraction of the distance migrated by the zone from the origin of a tracking dye.

CE = Carboxylesterase; ChE= Cholinesterase; CHsp = Cholinesterase like enzymes; ER= Esterases resistant to inhibitors; ArE = Arylesterases;

Esdp = Esterase sensitive to organophosphates and pCMB

Ese = Esterases sensitive to eserine alone;

+++ = High activity; ++ = Moderate activity; += Low activity; + = Very low activity;

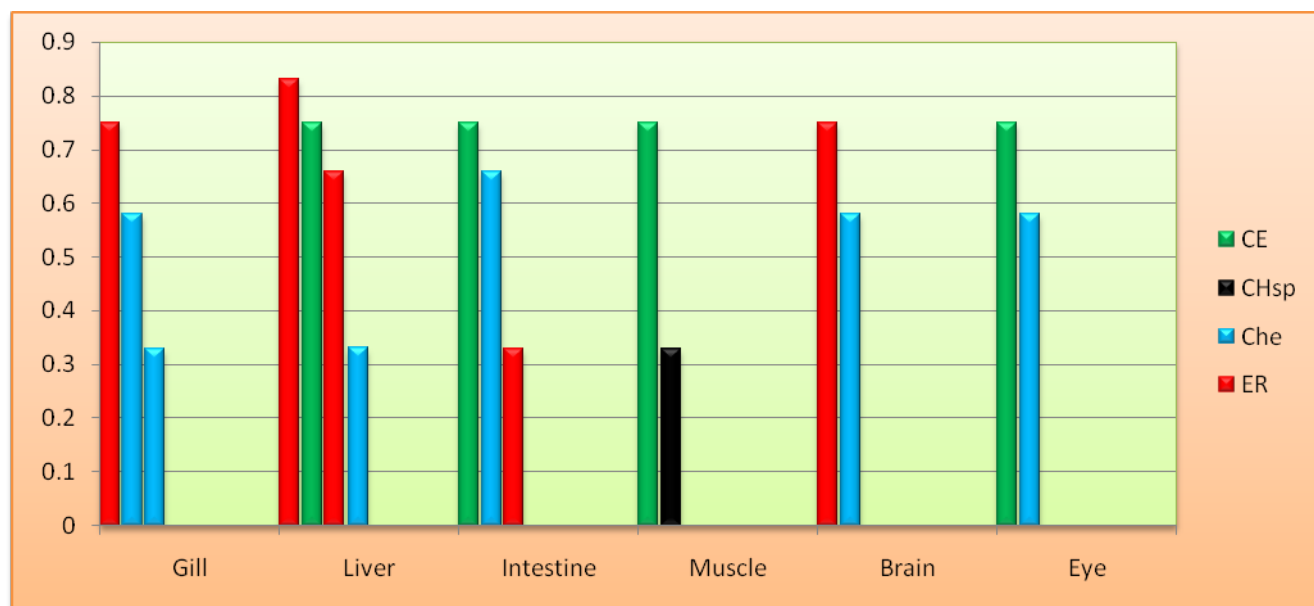
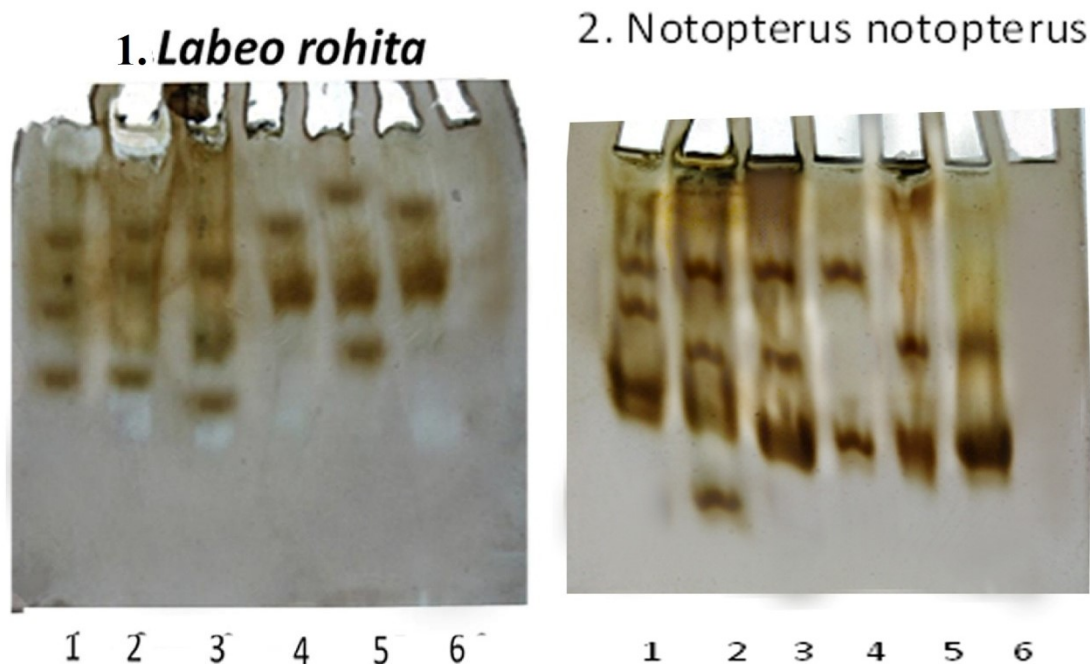
Figure 2. Tissue specific distribution of esterases in *Notopterus notopterus*


PLATE -I



Discussion

The present study demonstrated a considerable tissue specificity of esterase pattern in *Labeo rohita*. Five electrophoretically recognizable zones of esterases were observed with R_m of 0.58, 0.50, 0.41, 0.33 and 0.25. The multiplicity of the zones indicates the existence of different molecular esterase forms with different electrophoretic mobility and perhaps with different biochemical properties. A similar diversity of esterase forms was reported in different teleost fishes. Salamastrakis and Haritos observed 3 to 9 esterase bands in different tissues of *Trachurus trachurus*, with liver having a particularly high esterase diversity. Similarly, Gopalakrishnan et al. observed multiple esterase regions in liver, brain and muscle of *L. rohita* and *Cirrhinus mrigala*, indicating differences between the species and tissues.

Among the studied tissues of *L. rohita*, the R_m 0.50 zone had a relatively wide distribution, existing in gill, liver, intestine and brain. Interestingly, a similar electrophoretic zone was

characterized as different esterase types in different tissues: ER esterase in gill and intestine, CE esterase in liver and ChE esterase in brain. The fact demonstrates that identical or close migrating electrophoretic zones need not be identical biochemical enzyme types in different tissues. It means that tissue-specific modification or different expression of enzyme types can result in such differences. The previous research has also shown that electrophoretically identifiable esterase fractions may have a different substrate and inhibitor specificity in different tissues.

The existence of CE esterase in the liver of *L. rohita* is particularly interesting. The liver is an important metabolic and detoxifying organ. Carboxylesterases are involved in the hydrolysis of endogenous and xenobiotic esters. Several studies on fresh water fishes have demonstrated a particularly high esterase activity in digestive tissues (liver and intestine) and the existence of carboxylesterase forms, which can be involved in the detoxification process. Thus, the presence of CE esterase in the liver of *L. rohita* can reflect its metabolic and detoxifying functions.

Rm 0.41 zone was recorded in gill, muscle, brain and eye. CE esterase was detected in gill and eye, ChE esterase was present in muscle and ArE esterase was present in brain. Detection of ChE esterase in muscle and brain is justified by the known biological role of cholinesterases in nervous system and neuromuscular functions. In fishes, cholinesterases are present in various tissues with varying contributions depending upon tissue and fish species. Brain generally constitutes an important site for cholinesterase activity, while muscle also possesses high cholinesterase activity. The presence of ChE esterase in muscle and brain in the present study therefore seems biologically meaningful.

Rm 0.33 zone was present in liver, intestine, muscle and eye. CE, CHsp and ER esterases were present in the respective zones depending upon tissue. Presence of ER esterase in intestine and CE esterase in liver and eye suggests different enzymatic activities in these tissues. Esterases of intestine might be involved in digestion and metabolism of dietary chemicals, while esterases of liver may possess increased activity for metabolic transformation and detoxification purposes. Tissue specific presence of different forms of esterases has been reported in fishes, wherein liver and intestine show greater diversity of esterase activities than tissues such as gill and muscle.

Rm 0.25 zone was detected in gill, liver and brain. ER esterase was identified in gill and liver, while the same zone of brain contained ChE esterase. Presence of ER esterase in more than one tissue indicates that the inhibitor resistant forms of esterases may possess a relatively wide physiological distribution in *L. rohita*. Presence of different forms of esterases at same electrophoretic position highlights the fact that electrophoretic mobility is not sufficient to define enzyme. Inhibitor based assays and substrate specific assays have proven more effective for determination of enzyme identity in previous fish esterase studies.

Rm 0.58 zone was detected only in intestine and identified as Esdp esterase. Restricted expression

in intestine is highly significant as it suggests that the enzyme performs some specialized biochemical functions. Restricted expression of esterases in particular tissues has been reported extensively in teleosts and may serve as biochemical marker for the specific tissue or species.

Overall, the esterase profile of *L. rohita* suggests that CE and ER esterases are the major esterases, while ChE, ArE, CHsp and Esdp forms are present with restricted tissue distribution. Predominance of CE and ER forms suggests significance of metabolic,

Overall, the esterase profile of *L. rohita* indicates that **CE and ER esterases are the predominant forms**, while ChE, ArE, CHsp and Esdp forms occur with comparatively restricted tissue distributions. The predominance of CE and ER forms may reflect the importance of metabolic, digestive and detoxification processes in this freshwater fish. The marked differences in esterase composition among gill, liver, intestine, muscle, brain and eye also demonstrate strong tissue specificity.

Esterase distribution in *Notopterus notopterus*

The esterase pattern of *Notopterus notopterus* was also characterized by considerable tissue specificity. Five zones were detected at Rf 0.83, 0.75, 0.66, 0.58 and 0.33. Thus, although the number of zones was comparable to that observed in *L. rohita*, the tissue distribution and Rm values were significantly different. This difference indicates species-specific variation in the electrophoretic expression of esterase isozymes.

The Rm 0.83 zone occurred only in liver and was identified as ER esterase. The limited presence of this zone indicates a form of liver specific esterase in *N. notopterus*. In other fishes, liver-specific esterase expression has been reported and is normally linked to the intense metabolic and detoxification activity of hepatic tissue. In contrast, the **Rm 0.75 zone** was found in **all the tissues examined**, making it the most widely distributed esterase zone in *N. notopterus*.

However, its biochemical identity varied among tissues. It was classified as CE esterase in liver, intestine, muscle and eye, whereas it was identified as ER esterase in gill and brain. The broad distribution of this zone suggests that the corresponding esterase system may perform fundamental physiological functions in several tissues. At the same time, the tissue-dependent classification indicates that enzymes with similar electrophoretic mobility may have different substrate or inhibitor characteristics.

The widespread presence of CE esterase in liver, intestine, muscle and eye of *N. Notopterus* is especially interesting. Carboxylesterases are enzymes that break down ester-containing compounds and help in the metabolism of foreign substances. In fish liver and intestine carboxylesterases are linked to detoxification and metabolism. The Rm 0.66 zone was found in liver and intestine where Rm 0.66 zone was identified as ER esterase in liver and as ChE esterase in intestine. The narrow distribution shows that esterase types are expressed in tissues. The presence of ChE in intestine is also noteworthy because cholinesterases are not found in nervous tissue; different cholinesterase types can exist in peripheral tissues and may carry out both normal functions and protective roles. The Rm 0.58 zone appeared in gill, brain and eye and in all three tissues Rm 0.58 zone was identified as ChE. The appearance of ChE in brain is expected because ChE plays a role in nerve signalling while ChE is present in gill and eye showing that cholinesterase expression goes beyond neural tissues. Similar findings of ChE and pseudo-ChE, in brain, eye and muscle have been reported in teleosts.

The **Rm 0.33 zone** was detected in gill, liver, intestine and muscle. It was identified as ChE in gill and liver, ER esterase in intestine and CHsp esterase in muscle. This tissue-dependent variation further demonstrates the complexity of esterase expression in *N. notopterus*. The occurrence of CHsp esterase in muscle is of interest because muscle esterases, particularly cholinesterase-related forms, have been investigated as potential biochemical indicators of environmental exposure.

Comparative interpretation of the two species

A comparison of the two species demonstrates clear differences in their tissue-specific esterase profiles. *L. rohita* exhibited Rm values of **0.58, 0.50, 0.41, 0.33 and 0.25**, whereas *N. notopterus* showed **0.83, 0.75, 0.66, 0.58 and 0.33**. Only limited correspondence was observed between the two electrophoretic profiles. This difference in mobility patterns may reflect species-specific expression of esterase isoforms and could potentially be useful for biochemical identification and comparative genetic studies.

Such species-specific esterase patterns have been reported previously in fishes. Gopalakrishnan et al. demonstrated species-specific differences in esterase regions between *L. rohita* and *C. mrigala*. Similarly, esterase electrophoresis has been successfully used to distinguish fish species based on differences in the number and position of esterase bands. Therefore, the differences observed between *L. rohita* and *N. notopterus* in the present study support the usefulness of esterase electrophoresis as a biochemical taxonomic and comparative tool.

The present findings are also consistent with the broader application of allozyme electrophoresis in fish genetics. Esterase loci have been demonstrated to exhibit polymorphism in *L. rohita*, and Est-1 has been used as one of the loci for differentiating genetic stocks or ploidy groups. *N. notopterus* has also been shown to possess substantial genetic variation using allozyme and mitochondrial markers, indicating that this species contains useful biochemical and molecular variation for population studies.

From an ecological perspective, the tissue-specific occurrence of CE and ChE esterases is also important because these enzyme systems can respond to environmental contaminants. Cholinesterases, particularly AChE, are widely used as biomarkers of exposure to organophosphate and carbamate compounds, while carboxylesterases can participate in the metabolism and detoxification of xenobiotics. Consequently, the esterase profiles established in

the present study may provide a useful baseline for future investigations involving pesticide exposure, environmental stress and biochemical monitoring in these fishes.

In conclusion, the present study demonstrates that both *L. rohita* and *N. notopterus* possess **complex and distinctly tissue-specific esterase systems**. Although both species exhibited five electrophoretic zones, their Rm values, tissue distribution and biochemical classification differed considerably. *L. rohita* was characterized predominantly by CE and ER esterases, whereas *N. notopterus* showed extensive distribution of CE, ER and ChE forms with particularly broad expression of the Rm 0.75 zone. The observed interspecific and inter-tissue differences suggest that esterase isozyme patterns can serve as useful biochemical markers for **tissue characterization, comparative physiology, species discrimination and potentially population-genetic studies**. However, definitive assignment of individual bands to specific esterase classes should ideally be supported by standardized substrate-specific and inhibitor-based assays.

Conclusion

The present work compares the tissue specific esterase isoenzymes of two fresh water fish belonging to two different orders. Electrophoretic separation followed by inhibitor sensitivity tests showed considerable differences in the esterase profiles of these fish.

Five esterase zones were observed in *L. rohita* at Rm values 0.58, 0.50, 0.41, 0.33 and 0.25 while for *N. notopterus*, five esterase zones were observed at Rm values 0.83, 0.75, 0.66, 0.58 and 0.33. Both the fish species showed Rm values with equal number of zones in the range of 0.25–0.83, but the distribution pattern and the type of esterase present in these zones were found to be different. The esterase distribution in *L. rohita* and *N. notopterus* showed species specific pattern as evident from the differences in the esterase zones. The zone with Rm value 0.50 was found to be frequently distributed in most of the tissues

studied in *L. rohita*, while, in case of *N. notopterus*, the zone with Rm value 0.75 was the frequently distributed one.

The electrophoretic analysis showed that Carboxylesterases (CE) and inhibitor resistant esterases (ER) predominated in *L. rohita* while, in case of *N. notopterus*, all the three categories of esterase namely; CE, ER and Cholinesterase (ChE) showed almost equal distribution. Cholinesterases were found to be distributed in the brain and muscle of both the species which is in accordance with their role in nervous system functions. The presence of tissue restricted esterase was also evident from the study as shown by Esdp esterase of *L. rohita* in the intestinal tissue at Rm 0.58 and ER esterase of *N. notopterus* in liver tissue at Rm 0.83. Presence of cholinesterases in brain and muscle tissue showed their role in the nervous system functions and muscle coordination.

The most interesting observation was that of different esterase forms being present in the similar electrophoretic zones. Hence, it may be concluded that only on the basis of electrophoretic mobility, esterases cannot be identified, and their inhibitor sensitivity tests must be carried out for their proper identification.

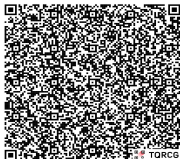
It may be concluded from the present study that *L. rohita* and *N. notopterus* exhibited a highly complex distribution pattern of esterase isoenzymes that varied with the tissue type. The present study demonstrated that esterase isoenzymes could be used as biochemical markers for taxonomic purpose. The baseline information on the esterase isoenzymes of these fish species may be useful in further investigations on the responses of these organisms to various environmental factors. It may be also noted that most of the esterases are sensitive to pesticides, and therefore, cholinesterases and carboxylesterases can be employed as biochemical markers for the detection of pesticide residues in the environment.

Thus, in the present investigation, it was observed that *L. rohita* and *N. notopterus* had different tissue specific esterase isoenzymes. These findings may provide useful baseline information on the isoenzyme distribution pattern in the two teleost fish species. The present findings also emphasize the need of carrying out further experiments for the proper identification of the esterase zones on the basis of their substrate specificities.

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